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Optical Assessment of OpenScope Objective Based on a Raspberry Pi Camera

Raspberry Pi Camera Specifications

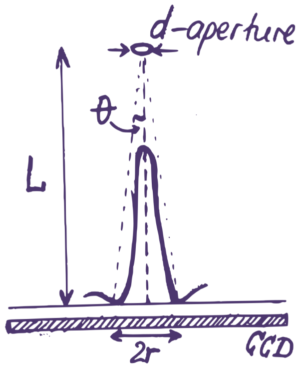
Property	Value
Pixel size	1.4μm $\times$1.4μm
Sensor size	2592px $\times$ 1944px
Total	5MP
Focal length	3.6mm
Aperture	1.25mm

Source: Raspberry Pi[1].

Theory of Optics

The resolution can be limited by two independent factors:

- pixel size that the light is magnified onto;
- diffraction effects.



If the light is under magnified onto the CCD, the CCD will determine the resolution; an over magnification will reduce the field of view as multiple pixels will represent the smallest resolvable point. In our case we know that the pixel size is 1.4 μm, so we now need to work out the diffraction limit, that is the smallest spot size which can be produced by the lens with the given specs. To calculate this, recall the Rayleigh criterion for a circular aperture:

$$\sin\theta = 1.22\frac{\lambda}{d}$$

Here λ ≈550nm is the wavelength of light, taking green for the middle of the visible spectrum, d=1.25mm is the diameter of the aperture and θ (small angle) is the angular radius of the spot, that is $\tan\theta = \frac{r}{L}$. Here, r is the radius of the spot projected at a distance L from the aperture, which in our setup is approximated by the focal length f of the Raspberry Pi camera lens (and the spot is projected onto the CCD sensor).

From first approximation for a small angle:

$$\sin\theta \approx \tan\theta \approx \theta$$

so $1.22\frac{\lambda}{d} = \frac{r}{f}$. Rearranging this equation and plugging in the numbers gives the following resolvable spot size 2r=3.8μm. By definition, the smallest resolvable distance between two objects is r. For the raspberry pi lens,

it is 1.9μm. This is greater than the CCD's pixel size, which imposes the actual limit on the resolution. The pixels of the CCD out resolve the theoretical lens limits for magnifications greater than 1.4/1.9= x0.74.

Final resolution estimate of a microscope based on Raspberry Pi camera: 1.9μm

Compare this with a typical size of a chloroplast: 5-8μm diameter [2]. Our resolution will be just enough to image them, which is exactly what we have managed to do on this picture of Spirogyra cells. Note that these are larger than typical chloroplasts though. To obtain a better resolution, a lens with either larger aperture and/or shorter focal distance can be used, without the need of a better CCD. However, this is a trade off in terms of worse aberration and contrast. An improvement to the resolution will however be required in order to image bacteria, for example, which are of the order of 1μm in diameter [3].

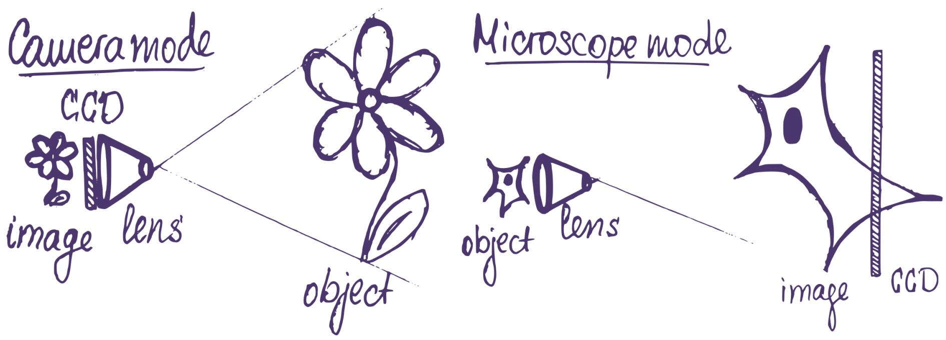
The Improvement

Ball lenses have very low spherical aberration and so focus light very accurately. They are available at a low cost and are simple to mount. We used a borosilicate (BK7) lens from Comar Optics (cat.no: 03 vQ 04, price 12.20 GBP) with focal distance 2.9mm and 4mm aperture. Analogous calculations give resolution of 0.4 μm for this lens. This enabled us to resolve bacteria (E.coli). Note that although sapphire lenses offer a shorter focal distance due to there greater optical index, their birefringent properties require plain-polarised light for a clear image.

Inverting the Pi Lens: Why and How

The way a camera works is by focusing an image of a distant large object as a small set of points onto the CCD, which is positioned close to the lens (in its focal plane). Theoretically however, it might as well do the opposite (because light paths are reversible – a well known and intuitive physical principle): that is, inspect the CCD pixels and project their greatly enlarged image onto a distant screen. The lens has a small aperture (1.25mm) at one end, and a larger one (4mm) offering a wider view angle at the other, which is required for viewing close up objects. This is normally oriented towards the CCD.

Figure 1: Inverting the lens



So, if we want to image a sample on a microscope slide, we need to:

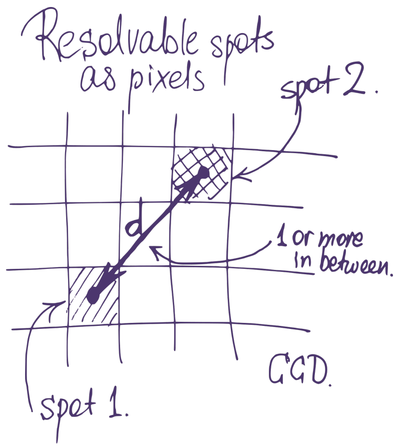
- position the lens close to the sample – at roughly the focal distance, that is 3.6mm;
- orient the lens with its larger aperture towards the sample;
- position the sensor behind the lens, now at a much larger distance (roughly 2.8cm) – for this we have designed a special camera mount.

Now we have the Raspberry Pi camera working as a microscope! The problem: how to unscrew the lens from the camera The Raspberry Pi camera is sold with the lens screwed (and lightly glued) into the sensor casing. To unscrew the lens, you will need the right type of pliers: ideally with ridged surface. Grip the top part of the plastic casing of the lens firmly, being careful not to crush it, and rotate counterclockwise. After the first small rotation, you should be able to unscrew it fully manually. ATTENTION: might not work from the first time! Do not try to cut out the lens or force it out in any other way.

Designing the objective

We have designed a 3D-printable objective for OpenScope [picture of cube]. The slot on the bottom face houses the CCD, and the circular opening on the opposite face – the lens. In designing the objective, it is crucial to position the lens at the correct distance from the CCD. This depends on the focal length, and the calculations to optimise the field of view and resolution proceed as follows:

We want the smallest resolution to correspond to the diagonal of three pixels, so that two spots that are resolvable have at least one pixel in between them.



If r is our smallest resolvable distance and w is the pixel width, then r must be magnified onto $2\sqrt{2}w$. Magnification, M is given by $M = v/u$ where u is the object distance and v is the image distance.

This gives us:

$$r \times M = \frac{rv}{u} = 2\sqrt{2}w \tag{1}$$

As the optics involved only contain one lens, we know that focal distance f, u, and v are related by:

$$\frac{1}{f} = \frac{1}{u} + \frac{1}{v} \tag{2}$$

Expressing Equation (2) as $\frac{1}{u} = \frac{v-f}{vf}$ and substituting this into Equation (1) gives:

$$\frac{rv(v-f)}{vf} = 2\sqrt{2}w \tag{3}$$

Asserting that $v \neq 0$ simplifies Equation (3) to:

$$\frac{r(v-f)}{f} = 2\sqrt{2}w$$

And so the image distance (distance from the CCD to the lens) is optimised at the distance:

$$v = \frac{2\sqrt{2}wf}{r} + f \tag{4}$$

Equation (4) can be generalised to n number of pixels separating the smallest resolvable distance:

$$v = f\left(\frac{\sqrt{2}(n+1)wf}{r} + 1\right) \tag{5}$$

To ensure clear images, at the cost of field of view, a value of $n = 3$ pixels was set as the optimum for our microscope.

With the Raspberry Pi lens v is then calculated to be:

$$v = (3.6)\left(\frac{\sqrt{2}(3+1)(1.4)}{1.9} + 1\right) = 18.6\text{mm}$$

A larger image distance would not affect maximum resolution but only reduce field of view.

As the .scad file containing the design of the objective is easily modifiable, users can change the distance from the CCD to the lens, so that it is optimized for the particular lens used.

References

[1] Wise, R. and Hoober, J. (2006). The structure and function of plastids. Dordrecht: Springer.

[2] Encyclopedia Britannica, (2015). bacteria :: Diversity of structure of bacteria. [online] [Accessed 30 Jul. 2015].